# CARDIOVASCULAR STUDIES ON COPPER-DEFICIENT SWINE

## IX. Repair of Vascular Defects in Deficient Swine Treated with Copper

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Vascular defects leading to rupture of the aorta and muscular arteries have been reported in copper-deficient swine[1,2] and chicks.[3-5] Histologic lesions of the elastic membranes similar to those in swine and chicks have also been reported in rabbits[6] and turkey poults[7] on copper-deficient diets. There is corresponding alteration in mechanical properties of affected aortas[8] that is, at least in part, a reflection of altered mechanical properties of the elastin.[9] Moreover, the elastin content,[10-13] solubility,[13] and its altered amino acid content[10-14] suggest a primary defect in this component.

Although the prevention of these defects by control diets of the same basic composition containing copper is explicitly demonstrated in some of the foregoing reports, the reversal of the defects by copper after the deficiency was established has not been documented. A prompt remission of severe anemia within 3 weeks of refeeding copper to deficient swine has been reported by Lahey and co-workers.[15] The fibrous repair of intimal fissures of the aorta in young copper-deficient swine treated with copper has been alluded to in a previous study.[16] The present study was undertaken to determine the extent of vascular repair and restoration of mechnical properties in vessels after reversal of copper deficiency in young swine.

### Experimental Methods

Several litters of mixed-breed swine, aged 2–4 days, were received at different times from the same breeder. They were divided into a copper-deficient group of 34 and a control group of 8 pigs. The management and diets have been described in detail in an earlier report.[13] In brief, the diet consists of sulfide-treated canned evaporated milk, supplemented with iron and trace elements, together with copper, 0.5 mg./kg./day, in the control animals. All animals were weighed and bled for measurement of volume of packed red cells at weekly intervals. Nineteen of the copper-deficient pigs were left untreated and of these, 12 died at 46–94 days and 7 were sacrificed at 50–84 days. The remaining 15 copper-deficient animals were left until the degree of anemia and physical signs indicated impending death, whereupon copper was added

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to their diet in the same dosage provided the control pigs. The treated animals were sacrificed at intervals of 10 days and at 2, 4, 5, and 6 weeks after beginning copper supplements. Their ages ranged from 59 to 131 days at time of sacrifice. The control animals were sacrificed at ages of 46–110 days. Aortic tensile strengths were measured routinely as described previously.[8] Segments from the coronary, splenic, hepatic, anterior mesenteric, pancreatic, renal, carotid, femoral, brachial, thoracic and abdominal aortic, and pulmonary arteries were fixed in Helly's fluid and after paraffin embedding were sectioned and stained with hematoxylin and eosin, and by the Verhoeff-van Gieson method for elastin.

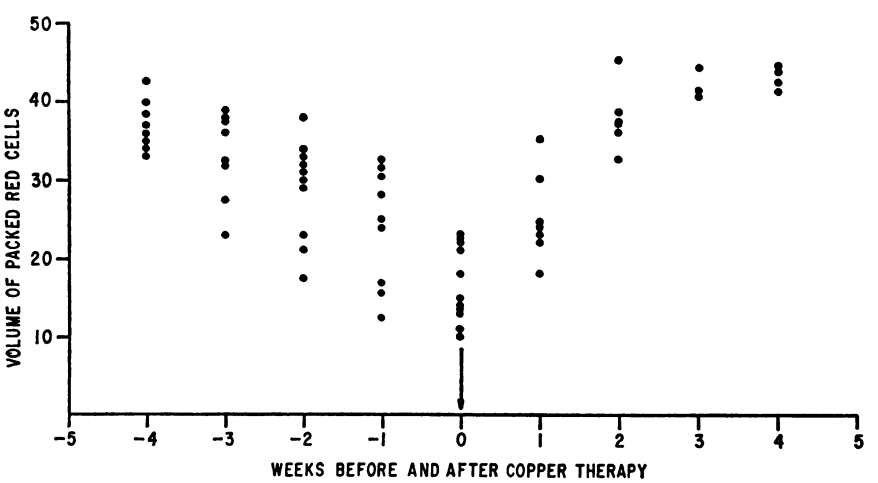
Selected samples for electron microscopy, taken from right and left main coronary arteries, were fixed in cold 3% glutaraldehyde in Millonig's phosphate buffer at pH 7.4 overnight, washed in the cold buffer, and trimmed to blocks less than 1 mm. in greatest dimension. The blocks were stored in the buffer variable periods of time and then postfixed in 1% osmium tetroxide in the same buffer, dehydrated in graded ethanol concentrations, cleared with propylene oxide, and embedded in Epon 812.® Ultrathin sections were stained with saturated uranyl acetate in 50% ethanol and examined with the Bendix Tronscope-TR50.®

## Results

The volume of packed red cells in the control pigs was 40–45% while it fell progressively in the deficient pigs to below 20% at death. At the time of reversal of the deficiency this value ranged between 10% and 21% in the copper-treated group. Following treatment there was rapid rise to normal levels by 3 weeks (Text-fig. 1).

The tensile strength of the control aortas was $16.5 \pm 2.8$ kg./sq.cm. while the aortas of the deficient animals varied between 2.4 and 5.1 kg./sq.cm. The aortic tensile strength of the treated group varied widely between 5.3 and 18.6 kg./sq.cm. (Text-fig. 2).

Text-figure 3 illustrates the response of aortic tensile strength to

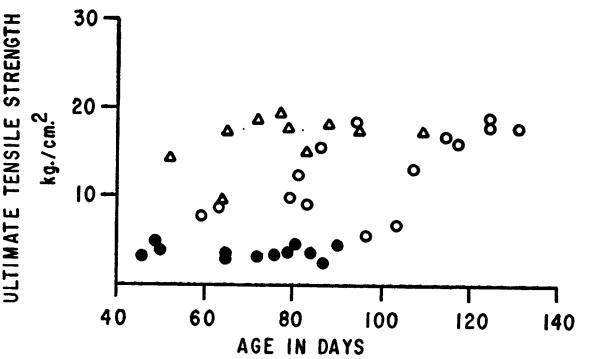



Text-fig. 1. Hematocrit readings at weekly intervals reflecting development of and recovery from anemia in copper-deficient pigs given copper supplements at time indicated by arrow.

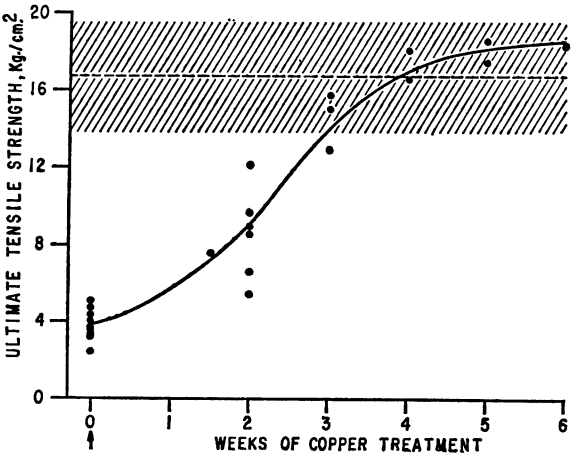
copper therapy as a function of time. After copper was added to the diet there was progressive strengthening, especially between the second and third weeks, leveling after the fourth week in the control range.

In the animals dying with copper deficiency, lesions were encountered similar to those previously reported.[1,2] Figure 1 illustrates the abdominal aorta of a deficient animal undergoing hemorrhagic dissection at the apex of the fissure. This process interrupts elastic laminas that are further fragmented and separated by smooth-muscle cells, collagen, and abundant amorphous matrix, some of which takes the Verhoeff hematoxylin stain for elastin. After 4 weeks of copper treatment, a similar aortic fissure still shows distorted and fragmented laminas, but the muscle cells are now plump and active, with a mesh of delicate elastin fibers in a pericellular distribution (Fig. 2). The excess of amorphous matrix is no longer visible.

In muscular arteries breaks frequently occur in the internal elastic lamina. Figure 3 illustrates changes in a splenic artery in established copper deficiency. The internal elastic lamina is interrupted at several




TEXT-FIG. 2. Tensile strength of aorta in control pigs (△), in pigs sacrificed when copper deficient (●), and in pigs that had been fed copper after development of deficiency (○), as function of age.




TEXT-FIG. 3. Tensile strength of aorta in pigs that were fed copper after establishment of deficiency. Dashed line and shaded area indicate mean and standard deviation of control values.

points. Repair of such lesions appears to be effected in 2 ways in the copper-treated animal. The first is a re-establishment of continuity by end-to-end formation of new elastin. Figures 4 and 5 illustrate part of a splenic artery from a pig fed copper for 4 weeks after severe deficiency had been established. The ends of the thick wavy elastica are joined by a fine, highly convoluted membrane of more recently formed elastin. In the second, there is formation of a new membrane parallel to the old. The hepatic artery in Fig. 6 is from an animal after 3 weeks of therapy. Internal to the original and fragmented elastica, there is a new, as yet incomplete lamina separated by radially oriented intimal cells. Duplication of the internal elastic lamina is occasionally seen in vessels from control animals, but the two membranes are always qualitatively identical and not associated with breaks in the lamina.

Electron micrographs of affected coronary arteries confirmed the nature of the new membranes staining with Verhoeff hematoxylin in the copper-treated pigs. Figure 7 shows a convoluted segment of thin membrane, similar to that in Fig. 4 and 5, joining the edges of a broken internal elastic membrane (outside the field of the picture). The irregular, nonfibrillar band of low density is surmounted on its intimal surface by a densely stained layer of unresolved structure. The excess of subendothelial amorphous reticulated substance and the space beneath the membrane contain sparse, densely stained collagen fibrils.

## Discussion

The rapid regeneration of elastic membranes in copper-deficient pigs refed copper in maintenance amounts reinforces earlier evidence that the defect in elastin in copper deficiency is specifically related to copper. It suggests further that the defect involves the biogenesis of elastin. Recent advances in the knowledge of the structure of elastin offer a plausible hypothesis to explain this defect as a consequence of the failure of certain oxidative steps in the biosynthesis of elastin.[17] The lowered content of desmosine and isodesmosine in elastin from copper-deficient animals,[12,18] which is accompanied by an increase of lysine,[11–13,18] suggests a block in the oxidative deamination of lysine that is postulated to be preliminary to the formation of desmosine cross-linkages. Several amine oxidases have been shown to be copper-containing enzymes.[19,20] This type of enzyme may be involved in the oxidation of lysine. The diamine oxidase of pig plasma is reduced to undetectable levels in copper deficiency and it is rapidly restored by refeeding copper.[21] A reduction in detectable free aldehyde groups of aortic elastin in copper deficiency[22] strengthens this hypothesis since aldehyde intermediates are expected from the oxidation of the lysyl groups to form cross linkages.[23]

The predicted effects of such a block in the biosynthesis of elastin upon the chemical and physical properties of the resultant product have been observed in experimental copper deficiency. The diminution in the percentage of insoluble elastin residue[9-12,18] may be due to a retarded formation of cross-linked elastin and to the solubility of its non–cross-linked precursor. The existence of this soluble precursor is postulated by analogy to the soluble precursors of collagen. Although a soluble protein of amino acid composition similar to that of elastin has been found in excess in the aortas of copper-deficient swine,[10] its relationship to elastin has not been established. The increased solubility of the insoluble elastin residue in formic acid[13] and its lowered tensile strength and elastic modulus[9] are consistent with a diminution in intramolecular cross-links.

The chief importance of the present work is the demonstration of the reversibility of the deficiency state and of the restoration of mechanical strength of the aorta coincident with the re-formation of elastic membranes during recovery.

## Summary

Copper deficiency was produced in young swine by weaning on a sulfide-treated, dilute, evaporated milk diet containing iron and other minerals and vitamins. Controls received a copper supplement. When severe deficiency was established, as judged by a fall in the volume of packed red cells and physical signs, some of the pigs were sacrificed and others were given copper supplements until sacrifice at intervals from 2 to 6 weeks later. There was prompt recovery from the anemia and from other signs of the deficiency, including tensile strength of the aorta. Coincidentally with the restoration of mechanical properties of the aorta during recovery there was repair of elastic membranes by new formation of elastin.

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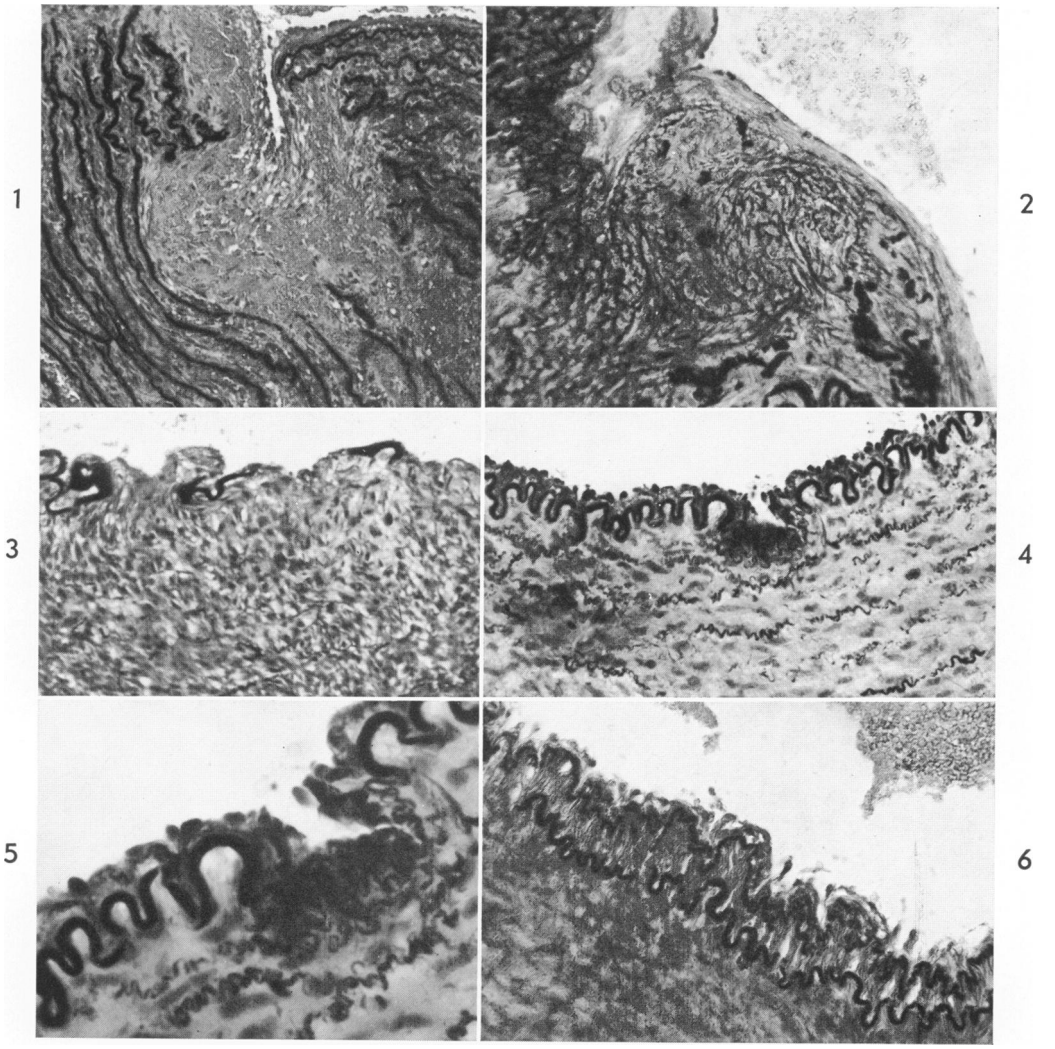


Sections illustrated in Fig 1–6 were stained with the Verhoeff-van Gieson stain.

FIG. 1. Aorta, deficient Pig 12, showing recent fissure of inner layers interrupting medial elastic laminas and filled with amorphous material. × 120.

FIG. 2. Aorta, Pig 24 treated with copper for 28 days following establishment of deficiency. Fissure has been filled in by cells and intercellular fibers that stain like elastin. × 190.

FIG. 3. Splenic artery, deficient Pig 22, showing interruptions of internal elastic lamina. × 90.

FIG. 4. Splenic artery Pig 1626 treated with copper for 28 days following establishment of deficiency. Interruption in original thick internal elastic lamina has been filled in by finer fibers or membranes staining like elastin. × 220.

FIG. 5. Detail of lesion in Fig. 7, showing elastin-staining thin fibers or membranes joining broken edges of original internal elastic membrane. × 485.

FIG. 6. Hepatic artery, Pig 23 treated with copper for 21 days following establishment of deficiency, showing duplication of internal elastic membrane. New membrane (above) is thinner and less regular than the old. × 240.

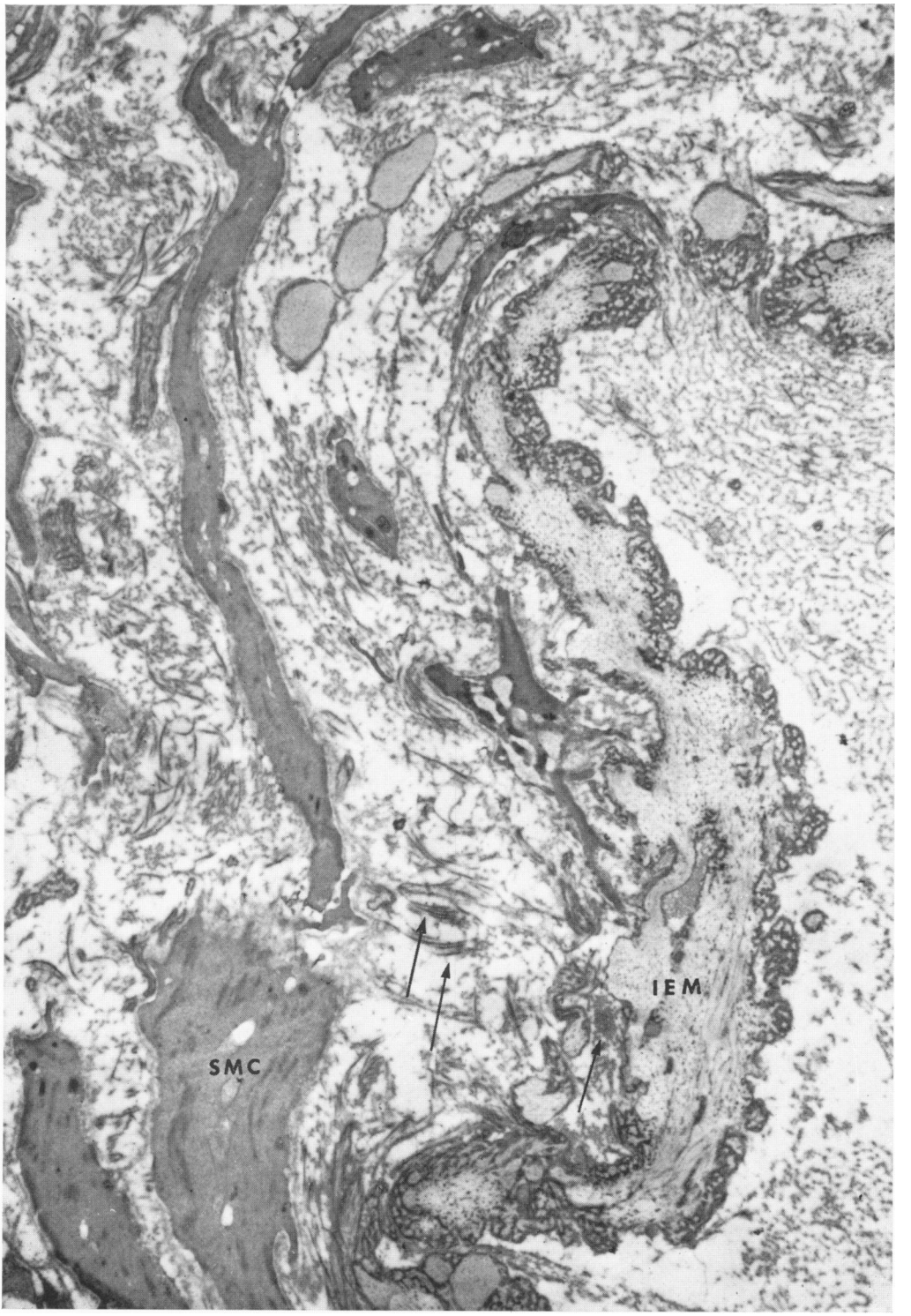


FIG. 7. Electron micrograph of coronary artery Pig 44 treated with copper for 11 days following establishment of deficiency. Re-forming internal elastic membrane (IEM), collagen fibers (arrows), and smooth-muscle cells of media (SMC) are shown. Uranyl acetate stain. × 10,000.